Toxicology Excellence for Risk Assessment

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December 22, 1997

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Dear Reviewers:

Progress is continuing on the perchlorate project. Both the 90-day study and the neurobehavioral developmental study are on schedule. The dose-range finding developmental study in rabbits is being started this week. This letter transmits draft protocols for the remaining tier of studies we are considering. This set of protocols includes a study of perchlorate's absorption, distribution, metabolism, and excretion; a study to characterize developmental effects; a study to provide data needed to reduce the uncertainty in extrapolation from animals to humans; and a study to identify possible biomarkers of perchlorate neurotoxicity. As you review these protocols, please consider whether these studies are complete and still relevant in addressing data gaps for perchlorate. In addition, the drinking water analytical chemistry SOP is enclosed for your information in response to earlier requests by several reviewers.

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Please send any comments you have on the draft protocols by mid January, 1998, if possible. You can reach me by phone at (606) 428-2744, by fax at (606) 428-3386, and by email at dollarhide@tera.org. Even if you have no technical comments, I would appreciate an email message letting me know that you found the protocols acceptable as is. As always, thank you for your time and contributions to this project.

Sincerely,

Joan S. Dollarhide
TERA

Enclosures

cc: C. Berrey, U.S. EPA Region IX
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Analysis of Perchlorate and Nitrate in Drinking Water

PURPOSE/PRINCIPLE:

This method is an ion chromatography method for the determination of perchlorate and nitrate in drinking water. A sample volume of 50 uL is introduced via a sample loop to the ion chromatograph where the anion of interest is chromatographically separated. The separated anion of interest is detected by a conductivity detector coupled with suppressed background. The analyte of interest is identified by their retention times and quantified by peak area. The practical quantitation limit is 0.005 ug/ml.

KEY WORDS: analytical chemistry, chromatography, ion chromatograph, drinking water, nitrate, perchlorate, conductive detector

1. SAFETY AND OPERATING PRECAUTIONS:

1.1. Ammonium perchlorate is a strong oxidizer and potentially explosive.

1.2. This method is restricted to analysts experienced in the nuances of ion chromatography and in the interpretation of the resulting ion chromatograms or personnel under supervision of such an analyst.

1.3. When analyzing unfamiliar samples; anion identification should be supported by the addition of spike solutions covering the analyte of interest.

1.4. Wear personal protective equipment: gloves, safety glasses, and laboratory jacket.

2. EQUIPMENT/MATERIALS:

2.1. Equipment.

2.1.1. Ion Chromagraph (IC). Ion chromatograph system is comprised of

2.1.1.1. Dionex autosampler, AI 350.

2.1.1.2. Dionex conductivity detector, CDM-3.

2.1.1.3. Dionex HPLC system, DX 300.

2.1.1.4. Dionex anion self regenerator.

2.1.1.5. IonPak AS-11 ion chromatography column and AG-11 guard column.

2.1.1.6. Dionex anion trap column, ATC-1.

2.1.2. Analytical Balance, capable of weighing to the nearest 0.0001g.

2.2. Materials.

2.2.1. 2 ml HPLC sample vials.

2.2.2. 0.1 and 1 L volumetric flasks.

2.2.3. 200 uL sample loop.

2.2.4. Weighing pan.

3. SPECIMEN/SAMPLE:

3.1. Sample Collection, Sample Preservation, and Storage.

3.1.1. Drinking water sample collection. Allow the water source to run freely for approximately 5-10 minutes. Collect duplicate samples in 2 ml HPLC vials for analysis.

3.1.2. Samples, blanks, and controls are stable when stored at 4-6 °C for up to two months without affecting the analytical results.

3.1.3. Samples, collected off-site, should be still can be shipped with packed ice by overnight delivery.

4. REAGENTS:

4.1. HPLC grade methanol or reagent grade methanol - demonstrated to be free of analyte.

4.2. 45 mM Sodium hydroxide, NaOH, prepared by dissolving 1.8 g of NaOH in 1 L 55:45 H_2O: Methanol.

4.3. 99.9% Ammonium nitrate granules, Aldrich chemicals, primary source.

4.4. 99.8% Ammonium perchlorate granules, Aldrich chemicals, primary source.

4.5. 99.9% Ammonium nitrate granules, Alpha chemicals, second source.

4.6. 99.9% Ammonium perchlorate granules, Alpha chemicals, second source.

4.7. De-ionized reverse osmosis water (DIRO H_2O).

4.8. Stock Standard Solutions.

4.8.1. Gravimetrically prepare approximately 50 mg/ml stock standard solution of ammonium nitrate and ammonium perchlorate in 1 L volumetric flask from pure neat standards purchased from primary and secondary sources using the following procedures.

4.8.1.1. Weigh out appropriate amount of neat stock chemical in a weighing pan. Transfer the chemical to a 1 L volumetric flask. Fill to 1 L with de-ionized reverse osmosis water.

4.8.1.2. Stopper and then mix by inverting the flask several times. Calculate the concentration in micrograms per milliliter (µg/mL) from the weight. When compound purity is certified at 96% or greater, the weight can be used without correction to calculate the concentration of the stock standard solution.

4.8.1.3. Store stock standard solutions in the same 1 L volumetric flask in a 4-6 °C refrigerator. The stock standard solution can be stored for up to 2 months.

4.9. Primary Dilution Standard Solution (Working Standard Solution).

4.9.1. Primary dilution standard solutions or working standard solution should be prepared from stock standard solutions.

4.9.2. The primary dilution standards should be prepared at concentrations that can be easily diluted to prepare calibration standard solutions (see Section 4.10) that will include the working concentration range. The recommended concentration for the primary dilution standards is 1000 ug/ml. Store the primary dilution standard solutions with minimal headspace. Consult section 4.2.2.4. for storage times.

4.9.3. Using equation 1, calculate the volume of the stock standard solutions needed to prepare 100 ml of the 1000 ug/ml primary dilution standard solution.

$$V = 1000 \text{ ug/ml} * 100 \text{ ml} / M \qquad \text{Equation 1.}$$

Where V and M are the volume and concentration of the stock standard solutions.

4.10. Calibration standards.

4.10.1. The number of calibration solutions needed depends on the calibration range. A minimum of three calibration standards is required to calibrate a range of a factor of 100.

4.10.2. To prepare a calibration standard, add an appropriate volume of a stock solution to a 100 ml volumetric flask and adjust the total volume to 100 ml with DIRO H_2O.

5. PROCEDURE:

5.1. Set the ion chromatograph operation conditions as followed:

Temperature	30 °C
Sample Volume	50 μL
Mobile Phase	45 mM NaOH in 55:45 water: methanol
Flow Rate	1 mL/min
Regenerant Flow Rate	10 mL/min

5.2. Initial demonstration of IC performance and analyst proficiency.

5.2.1. Initial Calibration. Follow IC conditions set forth in section 5.1. for analysis. Starting with the calibration standard of lowest concentration, analyze 50 μL of each calibration standard and produce a calibration curve by tabulating the area of peak of interest for each compound. If the ratio of peak area count to concentration is constant over the calibration range ($R^2 > 0.9990$), the average ratio can be used in place of a calibration curve.

5.2.2. Accuracy and Precision. Using IC conditions specified in section 5.1., analyze three to four replicates of a standard containing each analyte of concern at mid-concentration of the calibration range. See Section 6.4. and Section 7.5 for calculating and evaluating accuracy and precision.

5.2.3. Method Detection Limit. Using IC conditions specified in section 5.1., analyze at least seven replicates of a standard containing each analyte of concern at the lowest concentration of the calibration range. Consult Section 6.3.1. for calculating the method detection limit.

5.3. Routine IC analysis.

5.3.1. Reagent water blank. Reagent water blank consist of laboratory reagent water (de-ionize reverse osmosis water) should be analyzed according to conditions set forth in Section 5.1. Reagent water blank should be analyzed prior to running calibration standards and

samples to ensure that the reagent water blank is free from interference.

5.3.2. Calibration. Analyze and calibrate the IC system according to section 5.2.

5.3.3. Drinking Water Samples. Analyze samples by IC conditions set forth in Section 5.1. Identify and quantify unknowns by procedures described in Section 6.1. and 6.2.

5.3.4. Quality Control Check Standard (Control Standards). For each batch of ten samples, prepare a mid-level control standard and a reagent water blank. Analyze the control and water blank by using the chromatographic conditions set in section 5.1.

5.3.5. Spike Solution. Anion identification could be supported by the addition of spike solutions covering the analyte of interest. A 20 µg/mL spike solution can be prepared by injecting 50 µL of 400 µg/ml working stock standard solution into 1 mL of drinking water samples. See Section 7 for

5.3.6. Duplicate Analysis. One duplicate samples, chosen at random must be analyzed once every ten samples.

5.3.7. Concentration of stock standard, working stock standard and calibration standard solutions prepared from a primary chemical supplier must be verified by a check standard prepared from neat standards supplied by a secondary chemical supplier.

5.3.7.1. Follow Section 4.8, 4.9 and 4.10 for the preparation of stock standard, working stock standard and calibration standard solutions.

5.3.7.2. Perform this verification whenever a new or fresh stock standard solution is prepared from a primary chemical source.

6. DATA ANALYSIS/RECORDS:

6.1. Identify each analyte in the sample by comparing the retention time of the suspect peak to that generated by the calibration standards. Compounds should be identified as present when the criteria below are met. The retention time of the suspect peak should be within +/- 3% of the retention times of the standards.

6.2. The Concentration of the Unknowns.

6.2.1. Determine the concentration of the unknowns (C_u) by using the calibration curve or by comparing the peak area of the unknowns (A_u) to the peak area of the standards (A_s) as follows:

$$C_u \text{ (ug/ml)} = (A_u/A_s) \times C_s \qquad \text{Equation. 2.}$$

Where C_s is the known concentration of a standard.

6.2.2. Alternatively, if the linearity is greater than 0.995, the concentration of the unknown C_u can be calculated as followed:

$$C_u \text{ (µg/ml)} = (A_u) \times R \qquad \text{Equation. 3.}$$

Where R is the average ratio of area vs. concentration through the working calibration range.

6.2.3. Calculations should utilize all available digits of precision, but final reported concentrations should be rounded to an appropriate number of significant figures (three digit of uncertainty).

6.3. Method Detection Limits (MDL).

6.3.1. Analyze at least seven replicates of a standard containing each analyte of concern at the lowest concentration of the calibration range. Calculate MDL as followed:

$$MDL = t_c \times SD \times C/M \qquad \text{Equation. 4.}$$

Where:

t_c = students' t value appropriate for a 99% confidence level and a standard deviation estimate with n-1 degrees of freedom.

SD = standard deviation of the replicate analyses.

C = concentration.

M= mean area.

6.3.2. MDL for nitrate and perchlorate was determined when this SOP was written. The MDL was 0.005 μg/mL.

6.4. Accuracy and Precision.

6.4.1. Analyze three to four replicates of a standard containing each analyte of concern at a mid-range concentration.

6.4.2. For each analyte in each replicate, calculate the measured concentration, the mean concentration, mean accuracy as mean percentage of true value, and finally, the precision as relative standard deviation (RSD) of the measurements.

6.4.3. Acceptable criteria are outlined in Section 7.5.

6.5. Copy electronic data onto floppy disks and retain hard copies of all data (hard data) required by a specific SOP. The hard data retained will include copies of the sequence, method, chromatograms, calibration data and quantitation results. The storage location of the electronic and hard data will be stated in the laboratory notebook.

6.6. Reports.

6.6.1. Reports should be documented in the laboratory notebook and the reports should include the following:

6.6.1.1. Standard calibration curves for each analyte.

6.6.1.2. Method detection limits (MDLs).

6.6.1.3. Quantitation of the analytes found in the unknown samples, including blanks, control, etc. If not found, then report NA.

6.6.1.4. Precision and recovery data for each analyte.

6.6.1.5. Report any carry-over if found in reagent water blank(s).

6.6.1.6. Operating conditions.

6.6.2. Reports should also document samples and sampling conditions.

6.7. Record analysis details in project notebook along with copies of the report issued.

6.8. Record any analytical use, repair, and maintenance records in the instrument logbook.

7. QUALITY CONTROL:

7.1. The performance of the IC system and the proficiency of the analyst must be verified and validated by following steps outlined in Section 5.2. prior to sample analysis. Once the performance of the IC system and the proficiency of the analyst have been verified, follow step 5.3. for routine sample analysis.

7.2. The reagent water blank(s) must be demonstrated to be free from contamination. If interference is found in the chromatogram, rerun the reagent water blank. If the problem persists, replace cartridges in the de-ionize/reverse osmosis system and then re-run the reagent water blank. In the event that this fails use distilled water.

7.3. The retention times of the control standard must agree within +/- 3% of the retention times generated by the calibration standards. If the retention times show excessive drift, check for leaks.

7.4. Spike, Controls and Duplicate Analysis. If the response for any analyte varies from the predicted response by more than +/- 20%, the test must be repeated using either a fresh spike, calibration standard or duplicate sample. If the results still do not agree, generate a new calibration curve.

7.5. Accuracy and Precision. For each analyte, the mean accuracy, expressed as a percentage of the true value, should be 80-120% and the RSD should be less than 20%. Precision and accuracy data for both 0.05 µg/mL perchlorate and nitrate controls were obtained at the time when this method is written. Precision and accuracy data were 98.0% and 5.0 %, respectively.

7.6. Stock standard solution concentrations prepared from a primary chemical source must be verified by a secondary chemical source. The relative response difference between the two source should be less than 10%. If this criteria is not met, prepare fresh stock standard solution from both primary and secondary chemical and repeat the analysis.

8. LIMITATIONS OF PROCEDURE:

8.1. If sample concentration exceeds the calibration curve, dilute the sample by serial dilution and analyze it according steps described in Section 5.

9. PROCEDURE NOTES: N/A

10. REFERENCES:

10.1. EPA Method 300.0 Determination of Inorganic Anions by Ion Chromatography, Revision 2.1. August 1993

10.2. J. Glaser; D. Foerst, G. McKee, S. Quave, W. Budde. Method Detection Limit (MDL) as described in "Trace Analyses for Wastewater." *Environmental Science and Technology*, vol. 15, number 12, pp. 1426, Dec. 1981.

10.3. Private communication. Art Fitchett. Dionex Corp. Dated 17 April 1997.

AUTHOR: _____
GROUP ADMINISTRATOR: _____
QUALITY ASSURANCE: _____

10.4. Bulletin 882. Mobile Phases for Ion Exchange Chromatography and Chromatofocusing. SUPELCO.

10.5. Craig C. Williams. "Quality Control and Drug Analysis: Stability of Aqueous Perchlorate Formulations." *Amer. J. of Hospit Pharm.*, Vol. 34, pp. 93, 1977.

11. ADDENDA/ATTACHMENTS: N/A

AUTHOR: _____
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QUALITY ASSURANCE: _____